February 2025

Quick-Start Protocol

QIAprep&amp® Plasmodium Kit DBS Elution Workflow

The QIAprep&amp Plasmodium Kit (cat. no. 223213) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. Several components of the kit can be stored at room temperature (15–25°C): Blood Lysis Buffer, PR Buffer, DBS Wash Buffer. The S-Solution is stored light-protected at –20°C. This kit can be used for the detection of the Plasmodium parasite (in combination with the respective assay) in different workflows. The components used differ between workflows.

This workflow generates and assesses eluate from dried blood spots (DBS); it is intended to be used with dried blood samples collected on paper.

Further information

- *QIAprep&amp Plasmodium Kit Handbook*: **www.qiagen.com/HB-3663**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**
- *Pf/Non-Pf Detection Assay Kit Quick-Start Protocol*: **www.qiagen.com/HB-3669**
- *Pv/Pm/Po/Pk Detection Assay Kit Quick-Start Protocol*: **www.qiagen.com/HB-3671**
- *QIAprep&amp Plasmodium Kit Punching of Dried Blood Spots Quick-Start Protocol*: **www.qiagen.com/HB-3666**

Notes before starting

- The following blood collection cards are compatible for generating DBS: Whatman® filter paper (GE, cat. no. 3030-917 or 3017-915); QIAcard® Bloodstain card (cat. no. WB100014) or any other blood collection cards (untreated, no preservatives); as well as the QIAcard FTA Classic card (cat. no. WB 120205 or WB120305).
- It is recommended to use a puncher such as the Uni-Core® Punch 3 mm (cat. no. WB100078), or other comparable punchers.
- It is recommeded to use a Cutting mat (cat. no. WB100088).
- Thaw the QP&A DNA Mastermix, the PCR assay of choice, and RNase-free water.
- Additionally, this protocol uses DBS Wash Buffer, PR Buffer, and S-Solution.
    - The PR buffer forms a precipitate upon storage below 15°C. If necessary, redissolve by mild agitation at 37°C and then place at room temperature.
- Use the cycling conditions specified in this protocol.

Procedure

1. Vortex and centrifuge all reagents.
2. Take 1 paper punch from the blood sample collection card (preferably the middle of the blood spot) using a punching device (3 mm diameter of punch). Release the punch into the very bottom of a PCR plate/strip/tube.

   Note: Go to **www.qiagen.com/HB-3666** for a detailed instruction on how to use the puncher.

   Important: Depending on the applied pressure, pieces of the mat can be transferred into the reaction. This does not have a negative effect on the result, however, coring of the mat should be avoided.
3. Add 30 µL DBS Wash Buffer to the plate/strip tube. Close the tubes and/or seal the plate/strip tube thoroughly to prevent cross-contamination. If working in a plate, make sure to apply pressure uniformly on the adhesive foil across the entire plate, to obtain a tight closure around each well.

4. Vortex the plate/strip/tube for 10 s.
5. Centrifuge the plate/strip/tube for 3 min (3400 x *g*).
6. Carefully open the strip caps or remove the foil from the plate to avoid cross-contamination.
7. Remove and discard the DBS Wash Buffer from each vial or well using a pipette.

   Important: Do not dump out the liquid.
8. Add 60 µL of the PR Buffer into the plate.

   Note: Close the tubes and/or seal the plate/tube thoroughly to prevent cross-contamination. If working in a plate, make sure to apply pressure uniformly on the adhesive foil across the entire plate, to obtain a tight closure around each well. Centrifuge it briefly to collect the liquid at the bottom of the plate/tube.
9. Incubate for 10 min at 95°C in a PCR cycler (with heated lid). Cool the samples down to room temperature.
10. Centrifuge briefly and transfer 6 µL to a fresh plate well/strip/tube.

    Important: If you plan further tests, keep the remaining eluate from step 9. It is recommended to store the eluate at –20°C for up to 1 month.
11. **PCR setup**: Prepare the PCR reaction mix for a multiplex PCR reaction as shown in Table 1. Vortex briefly and centrifuge to remove the liquid from the cap.

Table 1. Reaction mix setup

Component	Channel for detection	1 rxn (µL)	Final Concentration
QP&A DNA Mastermix	–	9	1x
20x Assay Mix	Select respective channels	1	1x
S-Solution	–	2	–
RNase-free Water	–	2	–
Total volume	**–**	**14**	**–**

12. Add 14 µL of PCR reaction mix (Table 1) to the 6 µL eluate prepared in step 10.
13. Seal the plate/tube thoroughly with a fresh foil/lid. Mix gently by vortexing with medium pressure (5–10 s). Place the plate in different positions while vortexing, to ensure an equal contact with the vortex platform.
14. Centrifuge the plate/tube briefly to collect the liquid at the bottom of the plate/tube. Place it in the real-time cycler and start the cycling program (with heated lid). Program the cycler as referred to in Table 2.

    Note: Data acquisition should be performed during the annealing/extension step.

Table 2. Cycling conditions

Step	Time	Temperature (°C)	Ramp rate
PCR initial heat activation	2 min	95	Maximal/fast mode
2-step cycling (40 cycles)			
Denaturation	5 s	95	Maximal/fast mode
Combined annealing/extension*	30 s	58	Maximal/fast mode

*Add data acquisition

For general qPCR result interpretation, refer to the *QIAprep&amp Plasmodium Kit Handbook* at **www.qiagen.com/HB-3663**

Document Revision History

Date	Changes
02/2025	Initial release